

Application No. 10/511,527

Reply to Office Action

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AMENDMENTS TO THE CLAIMS

This listing of claims replaces all prior versions, and listings, of claims in the application.

1. (Currently Amended) Method for the ~~detection and~~ characterization of primary ~~tumors~~ ~~tumours~~ and ~~or~~ separate areas of primary ~~tumors~~ ~~tumours~~, respectively, the method ~~comprising~~ comprising:

(i) isolating or concentrating clusters of tumor cells contained in a sample material, wherein the sample material is selected from the group consisting of blood, urine, and nipple aspiration fluid from the female breast;

(ii) determining the genotype of polymorphic DNA sequences of microsatellite markers of the isolated or concentrated clusters of tumor cells contained in the sample material; and

(iii) characterizing the primary tumor or separate areas of the primary tumor according to the genotype of polymorphic DNA sequences. ~~using sample material to isolate and concentrate cell clusters of tumor cells, followed by an analysis of the genetic changes in these isolated cell clusters.~~

2.-3. (Canceled)

4. (Previously Presented) Method according to claim 1, wherein DNA of the following polymorphic sequences are analysed: D7S522, D8S133, D8S258, D8S265, NEFL, D10S541, D10S1765, D10S579, D13S153, D16S400, D16S402, D16S413, D16S422, p53, BB1, BB2, CAII, CAIII, CAIV, CAV and/or D17S855.

5. (Previously Presented) Method according to claim 1, wherein the polymorphic DNA is reproduced before analysis.

6. (Currently Amended) Method according to claim 5, wherein the polymorphic DNA of three polymorphic sequences, D7S522, D8S258, D16S400 or NEFL, D13S153, D17S855 or D10S541, D16S402, D16S422 are analyzed ~~analysed~~ together and/or reproduced.

7. (Previously Presented) Method according to claim 6, wherein the polymorphic DNA is reproduced prior to analysis by polymerase chain reaction (PCR).

Application No. 10/511,527

Reply to Office Action

8. (Currently Amended) Method according to claim 7, wherein the polymorphic DNA is reproduced by using the following primer pairs:
GCAGGACATGAGATGACTGA (SEQ ID NO: 1) and GTTATGCCACTCCCTCACAC (SEQ ID NO: 2) (for D7S522); GTTTGAAGAATTTGAGCCAACC (SEQ ID NO: 3) and TTCTTCTGCACACTTGGCAC (SEQ ID NO: 4) (for BB1+2); CTCGAGGTCTCATCTCTTTCC (SEQ ID NO: 5) and GCAGAGGTGCACAAAGGAGTAA (SEQ ID NO: 6) (for CAII); AGGCCACAGAGGAGATAACAG (SEQ ID NO: 7) and CAGGTGTGGTAGATGCCAAAGA (SEQ ID NO: 8) (for CAIII); GCAACTTATCCAAACCCTGACC (SEQ ID NO: 9) and AGAGTGGACTAGGAAATGCTAGGAG (SEQ ID NO: 10) (for CAIV); AGTTCTTGACTGGGAATTCGAT (SEQ ID NO: 11) and TTGGCCAAATTACACACCTTTG (SEQ ID NO: 12) (for CAV); TTCCATTTGTCTCGGTT (SEQ ID NO: 13) and AGTCTCCTCGTCTCACACCT (SEQ ID NO: 14) (for D7S2550); CAGTGCTGGAGTTGTTCAAG (SEQ ID NO: 15) and CTGGGAGTCAAGTGTTTTGG (SEQ ID NO: 16) (for D7S2429); TGCTAAGTCTTGATTTTGCC (SEQ ID NO: 17) and AACGGTCATCTGTGTTCG (SEQ ID NO: 18) (for D7S2467); GGTGTTTGTGTCATTACGCT (SEQ ID NO: 19) and TTTGCTGTAGAGGATGCAAT (SEQ ID NO: 20) (for D7S478); TTCGGGCTCTCTGTTATAAA (SEQ ID NO: 21) and CCGAAGCAGGATTTTATTTC (SEQ ID NO: 22) (for D7S670); AGCTGCCAGGAATCAACTGAGAG (SEQ ID NO: 23) and GATGCTCACATAAAGGAGGGAGG (SEQ ID NO: 24) (for D8S258); CCAATACCTGCAGTAGTGCC (SEQ ID NO: 25) and GAGCTGCTTAACACATAGGG (SEQ ID NO: 26) (for NEFL); CACCACAGACATCTACAACC (SEQ ID NO: 27) and CCAGTGAATAGTTCAGGGATGG (SEQ ID NO: 28) (for D10S541); AGGGTTATGTATAACCGACTCC (SEQ ID NO: 29) and GTCTAAGCCCTCGAGTTGTGG (SEQ ID NO: 30) (for D13S153); GGTTCACAATTGGACAGTAT (SEQ ID NO: 31) and GAACCCTCCATGCTGACATT (SEQ ID NO: 32) (for D16S400); GTACCCATGTACCCCAATA (SEQ ID NO: 33) and CAAAGCACCATAGACTAA (SEQ ID NO: 34) (for D16S402); GAGAGGAAGGTGGAAATACA (SEQ ID NO: 35) and GTTTAGCAGAATGAGAATAT (SEQ ID NO: 36) (for D16S422); AATAAATCCCACTGCCACTC (SEQ ID NO: 37) and ATCCCCTGAGGGATACTATTC (SEQ ID NO: 38) (for p53); GGATGGCCTTTTAGAAAGTGG (SEQ ID NO: 39) and ACACAGACTTGTCTACTGCC (SEQ ID NO: 40) (for D17S855).

9. (Currently Amended) Method according to claim 5, wherein the reproduced DNA fragments are split and analyzed ~~analysed~~ by capillary electrophoresis.

Application No. 10/511,527

Reply to Office Action

10. (Currently Amended) Method according to claim 1, wherein the ~~isolation or concentration of tumour~~ tumor cells isolated from the sample material are cytokeratin-positive cells ~~were isolated from sample material~~, and/or positive epithelial cells positive for ~~tissue-specific~~ tissue-specific proteins.

11. (Currently Amended) Method according to claim 10, wherein isolated epithelial cells are concentrated from the sample material by ~~means of~~ density gradient centrifugation, ~~centrifugation if necessary after homogenisation in a solvent~~, and wherein isolated cytokeratin-positive and/or positive epithelial cell clusters positive ~~from~~ for tissue specific proteins are ~~then~~ split off by ~~means of~~ immunomagnetic cell isolation.

12. (Currently Amended) Method according to claim 11, wherein ~~the medium for~~ the density gradient centrifugation is carried out using a hyper-osmotic medium.

13. (Currently Amended) Method according to claim 12, wherein the hyper-osmotic medium ~~buffer~~ consists of one of the following mediums: 13.8% (w/v) Diatrizoate and 8% (w/v) dextran 500 in H₂O (~~polymorphprep~~) or 13% (w/v) Nycodenz, 0.58% (w/v) NaCl and 5 mM Tricine-NaOH pH 7.4 in H₂O, ~~H₂O (Nycoprep)~~.

14. (Currently Amended) Method according to claim 1, wherein genetic changes in the isolated cell clusters are analyzed ~~analysed by means of~~ cluster analysis.

15. (Currently Amended) ~~Application of a method~~ Method according to claim 1, further comprising determining the tumor development ~~1 for the molecular characterization of tumours or tumour sections or for the determination of clonality from cells clusters isolated from sample material as well as for the detection of a tumour to determine the tumour stage, the metastasising~~ metastasizing potential, therapy requirements, efficacy of therapy of a tumour ~~tumor~~ or part thereof, ~~as well as the assessment of~~ or assessing the course of a disease or therapy.

16. (Currently Amended) ~~Application~~ Method according to claim 15, wherein the tumor cells or separate areas of tumor cells are from 15 for the detection and/or characterisation of tumours or tumour areas of the following carcinomas: mamma-, ovarial-, colon-, gastric-, prostate and/or bladder carcinoma.

Application No. 10/511,527

Reply to Office Action

17. (New) Method according to claim 5, wherein the polymorphic DNA of D7S522, D8S258, D16S400 are analyzed together and/or reproduced.

18. (New) Method according to claim 7, wherein the polymorphic DNA is reproduced by using the following primer pairs:
GCAGGACATGAGATGACTGA (SEQ ID NO: 1) and GTTATGCCACTCCCTCACAC (SEQ ID NO: 2) (for D7S522); AGCTGCCAGGAATCAACTGAGAG (SEQ ID NO: 23) and GATGCTCACATAAAGGAGGGAGG (SEQ ID NO: 24) (for D8S258); and GGTTCAACAATTGGACAGTAT (SEQ ID NO: 31) and GAACCCTCCATGCTGACATT (SEQ ID NO: 32) (for D16S400).